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Comparison of Four Enzyme Immunoassays for the Detection of Immunoglobulin G Antibody Against Glomerular Basement Membrane

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Goodpasture syndrome is a life-threatening autoimmune kidney and pulmonary disease that is characterized by pulmonary hemorrhage, renal failure, and the presence of autoantibodies against glomerular basement membrane (GBM) by indirect fluorescent antibody (IFA) techniques. In 1988, these antibodies were found to be specific for the noncollagen region of the α3 collagen IV chain. The antigen is characterized by a restricted tissue distribution occurring mainly in the kidneys and lungs. Specific enzyme immunoassays (EIAs) for anti-GBM have now been developed for in vitro diagnostic use in the laboratory. Our objective in this study was to compare the results obtained using four different EIAs for detecting anti-GBM IgG antibody with those using the standard IFA method using tissue from human kidney. Thirty-two patients with suspected Goodpasture syndrome, and 10 control sera were included in the study. GBM EIAs were purchased from or donated by the following vendors: Scimedx Corporation (Denville, NJ), INOVA Diagnostics (San Diego, CA), The Binding Site (Birmingham, England), and Wieslab (distributed by DiaSorin, Inc., Stillwater, MN). Percent agreement, sensitivity, and specificity were calculated for each EIA as compared to IFA. The results were as follows: Binding Site: 83.3, 100.0, and 72.0%; Wieslab: 95.2, 94.1, and 96.0%; INOVA: 96.2, 100.0, and 92.3%; and Scimedx: 81.0, 100.0, and 68.0%. We conclude that the INOVA and Wieslab GBM IgG EIAs compared well with the standard GBM IFA method using tissue from human kidney. The Scimedx and Binding Site EIAs had eight and seven false-positive results, respectively, when compared to GBM IFA using human kidney. The authors recommend conducting thorough evaluations of EIAs that screen for anti-GBM antibody before their implementation in the clinical laboratory. We also recommend confirming GBM EIA-positive sera by the standard IFA method using tissue from human kidney. J. Clin. Lab. Anal. 16:143–145, 2002.

Key words: glomerular basement membrane (GBM); Goodpasture syndrome; IgG; autoimmune kidney disease; enzyme immunoassay (EIA); indirect fluorescent antibody (IFA)

INTRODUCTION

Goodpasture syndrome is a hypersensitivity disorder of unknown cause, characterized by circulating autoantibodies against the glomerular basement membrane (GBM), resulting in pulmonary hemorrhage along with severe and progressive glomerulonephritis. The Goodpasture antigen (1) is found mainly in tissues of the kidneys and lungs (2–4). Less than one third of patients with renopulmonary syndromes will have antibodies against GBM, with the majority having autoantibodies to myeloperoxidase (MPO) or proteinase-3 (PR-3), as found in patients with Wegener's granulomatosis and Churg-Strauss syndrome (5,6). It is important for the clinician to determine the specific cause for the underlying renopulmonary syndrome for treatment purposes. Indirect fluorescent antibody (IFA) techniques employing monkey or human kidney tissue as substrate are used to detect and titer antibodies against GBM (7–10). Recently, commercial enzyme immunoassays (EIAs) have become available that detect specific antibodies against the Goodpasture antigen (α3 collagen IV chain). Our

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objective in this study was to compare these specific EIAs to an IFA using tissue from human kidney, and to determine the ability of the EIAs to screen for antibodies against GBM.

MATERIALS AND METHODS

Clinical Sera

Sera from 32 patients suspected of having Goodpasture syndrome, and 10 normal blood donors were included in the study. All sera were stored at 2–8°C until all testing was complete.

Anti-GBM by IFA

All anti-GBM IFA testing was performed by laboratory personnel of the Department of Pathology, University of Utah School of Medicine, Salt Lake City, Utah. Thin sections of human kidney fixed to glass slides were treated with a urea buffer before incubation at room temperature with diluted patient sera for 30 min. Slides were then rinsed and allowed to soak for 5 min in PBS buffer. FITC-labeled antihuman IgG conjugate was applied and allowed to incubate for 30 min at room temperature. Slides were then washed as before and cover slips were mounted using a glycerin-based mounting medium. Slides were then viewed at 400× using a fluorescent microscope. Positive sera show at least 1+ or greater fluorescence of the GBM at an IFA titer of 1:5.

Anti-GBM by EIA

Each manufacturer claimed to detect IgG antibody against the Goodpasture antigen (α3 collagen IV chain), and kits were purchased or donated by the following: Scimedx Corporation (Denville, NJ), INOVA Diagnostics (San Diego, CA), The Binding Site (Birmingham, England), and DiaSorin, Inc. (Stillwater, MN). All procedures were followed precisely according to the product insert.

Statistical Analysis

For calculating agreement, sensitivity, and specificity, the following formulas were used: A = sera with positive results by both the standard IFA and EIA assays; B = sera with negative results by the standard IFA, and positive results by EIA; C = sera with positive results by the standard IFA, and negative results by EIA; and D = sera with negative results by both the standard IFA and EIA assays. Agreement was equal to (A + D)/(A + B + C + D), sensitivity was equal to A/(A + C), and specificity was equal to D/(B + D).

RESULTS

When compared to IFA using tissue from human kidney, these EIAs obtained the following percent agreement, sensitivity, and specificity, respectively: Binding Site: 88.1, 100.0, and 80.0; Wieslab: 95.2, 94.1, and 96.0; INOVA: 96.2, 100.0, and 92.3; and Scimedx: 81.0, 100.0, and 68.0 (Table 1). The INOVA, Binding Site, and Scimedx EIAs gave two, seven, and eight false-positive results, respectively, when compared to IFA (samples 1–4 and 6–11; Table 2). The majority of these discrepant sera generated EIA values slightly above the cut-off, but some seemed grossly discrepant (samples 1, 4, 6, 7, and 9 for Scimedx, and sample 9 for Binding Site; Table 2). Discrepant sera were repeated a second time by IFA and EIA(s). All EIAs met the required quality control as set by the manufacturer. Positive results were obtained from three or more EIAs on two sera that were negative by IFA (samples 4 and 10; Table 2). Sample 4 (Table 2) showed a strong signal (high value) when using the Scimedx and Wieslab EIAs, but was only weakly positive by the INOVA and Binding Site EIAs. Sample 10 (Table 2) generated weakly positive values on the Scimedx, INOVA, and Binding Site EIAs, but gave negative results with the Wieslab EIA. These two sera (samples 4 and 10; Table 2) gave negative results a second time when repeated by anti-GBM IFA using human kidney. Only one false-negative result was noted when using the Wieslab EIA (sample 5; Table 2). Sample 5 (Table 2) gave negative results of 9.4 (cut-off = 10.0) repeatedly when using the Wieslab EIA, while all other EIAs gave values indicating the presence of high levels of anti-GBM antibody. Sample 5 (Table 2) was repeated on all EIAs as well as IFA to ensure accurate results. Sample 5 had an endpoint anti-GBM IFA titer of 1:320.

TABLE 1. Percent agreement, sensitivity and specificity of four enzyme immunoassays when compared to GBM IFA using human kidney

	Scimedx	INOVA	Binding Site	Wieslab
% Agreement	81.0	96.2	83.3	95.2
% Sensitivity	100.0	100.0	100.0	94.1
% Specificity	68.0	92.3	72.0	96.0

TABLE 2. Results from Anti-GBM IFA and EIAs in 11 discrepant sera

	GBM IFA	Scimedx EIA[a]	INOVA EIA[b]	Binding Site EIA[c]	Wieslab EIA[d]
1	–	16.3	5.0	1.9	9.6
2	–	4.6	3.4	4.9	8.9
3	–	5.8	3.7	1.8	4.2
4	–	15.4	29.0	4.9	157.0
5	+	28.8	113.0	25.6	9.4
6	–	10.5	11.2	4.5	1.9
7	–	15.6	2.7	2.1	1.3
8	–	5.9	7.7	3.1	0.9
9	–	14.7	12.2	20.9	6.4
10	–	5.6	27.7	7.7	3.5
11	–	1.7	3.3	5.4	1.0

[a]Interpretation of anti-GBM units (EU/mL) are as follows: < or equal to 5.0 = negative; > 5.0 = positive.
[b]Interpretation of anti-GBM Units are as follows: 0–20 = negative; 21–30 = weak positive; > 30 = moderate to strong positive.
[c]Interpretation of anti-GBM U/mL are as follows: < or equal to 3.0 = negative; > 3.0 = positive.
[d]Interpretation of anti-GBM Units are as follows: < 10 = negative; 10–20 = equivocal; > 20 = positive.

DISCUSSION

In any screening assay, the main goal is to achieve optimal sensitivity and specificity against the current standard method. It is extremely difficult to attain 100% correlation between two assays when comparing such drastically different methods as EIA and IFA. Although none of these anti-GBM EIAs correlated perfectly with IFA, the INOVA and Wieslab assays performed well in screening out IFA-negative sera (Table 1). We were unable to resolve the two Wieslab discrepant sera (samples 4 and 5; Table 2). These samples were repeated multiple times by both EIA and IFA, as well as by different technicians, to ensure accurate results. Correct sample order was also checked and maintained throughout the study. In the case of sample 5 (Table 2), the possibility of a prozone effect (excess specific antibody) was eliminated by titering the sample (1:100–1:128,000) using the Wieslab EIA and obtaining negative results on all eight titers. The development of any screening assay should incorporate the possibility of a prozone effect by ensuring a wide range of detection of specific antibodies at various levels. In general, the specificity of an EIA that utilizes a single defined antigen is higher than that of methods possessing other antigens in addition to the target antigen (i.e., IFA). The Binding Site and Scimedx EIAs both demonstrated poor specificity when compared to anti-GBM IFA using human kidney (Table 1). These two EIAs (Scimedx and Binding Site) utilized a lower serum dilution (1:50) than the other two EIAs (INOVA and Wieslab; 1:100). The lower specificity observed in these two EIAs may be due to excess serum antibody, other interfering substances, or an inadequate amount of specific antigen in the assay. Often one can maximize the use of an expensive antigen by lowering the initial serum dilution (increasing antibody concentration) in the system without compromising sensitivity or specificity. Sometimes the deficiency in antigen is not recognized until after the test has been implemented in the clinical laboratory. In our opinion, these anti-GBM EIAs are not diagnostic by themselves and all EIA positive sera should be confirmed by IFA, preferably using human kidney. Moreover, anti-GBM EIAs should only be used for patient screening and following the treatment of patients that have been confirmed positive by IFA.

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